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14. ABSTRACT

Precursor (Pre) T cells, when adoptively transferred further develop in the thymus into mature T cells, that is capable of lysing tumors. The overall objective of this project is to develop an effective 'off the shelf' adoptive cell therapy with PreT cells targeting prostate cancer. To enhance the anti-tumor activity of PreT cells we transduced these cells with a chimeric antigen receptor (CAR)-Pz1 targeting the prostate cancer associated antigen - prostate specific membrane antigen (PSMA). Here we show that prostate cancer specific PreT cells can be generated by expressing CARs targeting human PSMA. T cells derived from the adoptively transferred PreT cells develop in thymic and extrathymic sites and prolong survival of prostate cancer bearing recipients.

15. SUBJECT TERMS

prostate cancer, precursor T cells, adoptive transfer, prostate specific membrane antigen, chimeric antigen receptor

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Table of Contents

1. INTRODUCTION

Broader use of individual cell therapies are restricted by complex and expensive processes to manufacture a cell product that is limited to single patient use. The *goal of this project* is to extend the possibilities of curing malignant states using the immune system. *The overall objective of this application* is to develop an effective 'off-the-shelf' T cell precursor-based therapeutic strategy targeting prostate cancer. Based on studies in our and other laboratories we have *hypothesized* that genetically engineered T cell precursors (preT) expressing chimeric antigen receptors targeting prostate specific membrane antigen (PSMA) [1] can be adoptively transferred to enhance tumor killing in prostate cancer-bearing recipients. We are developing an "off-the-shelf" prostate cancer cell therapy using preT cells with specific anti-PSMA activity, which can be administered after non-myeloablative conditioning and can engraft both in thymic and extrathymic sites without risk for graft-versus-host disease.

2. BODY

2.1 Development of hPSMA-specific T cell precursors.

T cell precursors can be engineered to stably express a chimeric antigen receptor (CAR) using viral vectors (Figure 1). The Pz1 expression lentiviral vector was designed in our collaborating laboratory led by Dr. Michel Sadelain. T cell precursors were developed *in vitro* from C57BL/6 BM-derived HSCs using the OP9-DL1 culture system. Viral vectors were produced by tripartite transfection of 293T cells with transfer genes (Pz1), pCMVΔR8.92, and pUCMD.G[53] using 293 Transit (Mirus Bio). Vector supernatants were concentrated by ultra-centrifugation and 0.75-1.5 x 10^8 total TU used to transduce 5 x 10^5 T cell precursors (co-culture day 4–6) over 2 days in tissue culture plates coated with retronectin. Transduced cells were then expanded for an additional 14-21 days by OP9-DL1 co-culture. Transduction efficiencies of the T cell precursors were determined by flow cytometric analysis and were routinely in the range of 60% (Figure 2). FACS analysis of the co-cultured preT cells demonstrated primarily a double negative (DN) 2 and 3 stage characterized by CD4-CD8- CD44+/-CD25+.

2.2 Pz1+ preT cells home into thymic and extrathymic locations.

We adoptively transferred Pz1+ preT cells in B6→ B6 syngeneic bone marrow transplantation recipients (BMT) recipients. Pz1+ viral vector containing luciferase gene was used to track the transduced preT cells using in vivo imaging system (IVIS) [2]. Weekly imaging of the transplanted mice demonstrated that the transduced Pz1+ preT cells traffic to the thymus for further development (Figure 3). Pz1+ preT cells are also seen to migrate to extrathymic sites. FACS analysis of the adoptively transferred mice showed mature Pz1+ T cells derived from adoptively transferred preT cells in host spleens (data not shown). Similar trafficking was also seen in control 19z1+ preT cells, indicating that the expression of the CAR Pz1 does not affect the trafficking and development of preT cells.

Further investigation revealed that preT cells engraft in extrathymic sites including gut and mesenteric lymph nodes. To study the development of extrathymic T cells from ex vivo-generated preT cells, we adoptively transferred preT cells in irradiated nude mice. Nude mice lack the *Foxn1* gene and do not have a functional thymus. We found that mature T cells derived from ex vivo-generated preT cells can be obtained in the absence of a functional thymus. These T cells can produce IFNγ and TNFα following stimulation. However, total body irradiation was critical to the engraftment and a single dose of 600cGy led to engraftment and T cell development (data not shown). We are currently exploring the development of T cells in aged mice, where the thymic function is known to decrease. These studies are relevant to patients of prostate cancer undergoing adoptive transfer of preT cells, who are usually of the older age group with a deficient thymic state.

2.3 Pz1+ preT cells can enhance survival of prostate cancer-bearing recipients.
We adoptively transferred Pz1+ preT cells in B6→ B6 syngeneic BMT recipients bearing hPSMA+RM1 tumor. Survival was monitored. Significantly enhanced survival of tumor-bearing mice was observed in the group treated with Pz1+ preT cells compared to those treated with control 19z1+ preT cells or the untreated group (Figure 4). Our results show that adoptive transfer of Pz1+ preT cells can immunologically target hPSMA-expressing cells and improve survival in tumor-bearing recipients. We are currently exploring the effect of third party Pz1+ preT cells in tumor-bearing hosts undergoing syngeneic BMT or non-myleoablative conditioning.

3. KEY RESEARCH ACCOMPLISHMENTS

1. We developed hPSMA-specific preT cells for adoptive transfer.
2. We demonstrated that Pz1+ preT cells undergo further development in the thymus and give rise to mature Pz1+ T cells.
3. Adoptively transfer of preT cells demonstrated that they also traffic to extrathymic sites and undergo further differentiation into mature T cells.
4. Adoptive transfer of Pz1+preT cells improved survival in prostate cancer-bearing recipients.

4. REPORTABLE OUTCOMES

-None-

5. CONCLUSION

In the present study, we extend our previous findings that Pz1+ preT cells can be generated for adoptive transfer. We also show that adoptively transferred Pz1+ preT undergo further differentiation in the thymus to give rise to Pz1+ mature CD4+ and CD8+ T cells. We also found that adoptively transferred preT cells can traffic to extrathymic locations to develop into functional mature T cells. We then adoptively transferred Pz1+ and control 19z+ preT cells into hPSMA+tumor-bearing recipients. Groups treated with Pz1+ preT cells had significantly better survival in tumor-bearing recipients.
Implications and outlook: Within the planned schedule, we have worked towards developing chimeric antigen receptor-expressing precursor T cells that can target prostate-specific membrane antigen-expressing tumor cells. Having proved the feasibility of this approach we will now adapt this approach using non-myeloablative conditioning regimens that we have developed over the last year. This would lead to the development of an “off-the-shelf” cell-based therapy without the requirement of bone marrow transplantation.

6. REFERENCES

1. Gade, T.P., *et al.* Targeted elimination of prostate cancer by genetically directed human T lymphocytes. *Cancer Res* **65**, 9080-9088 (2005).
2. Santos, E.B., *et al.* Sensitive in vivo imaging of T cells using a membrane-bound Gaussia princeps luciferase. *Nat Med* **15**, 338-344 (2009).

7. SUPPORTING DATA

Figure 1

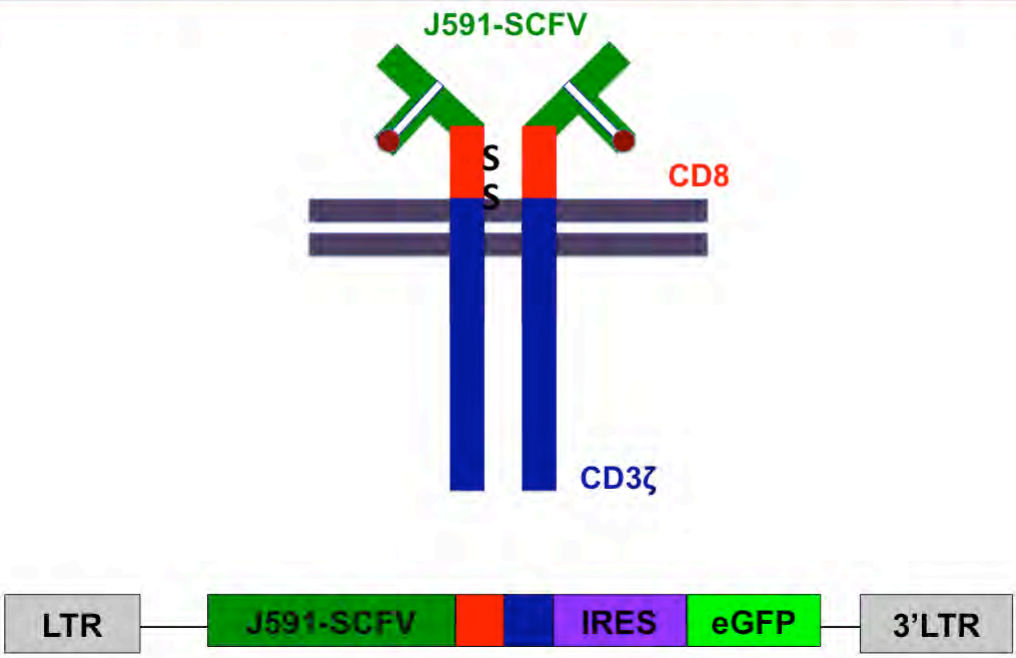

Figure 1: Pz1 – Chimeric Antigen Receptor targeting human Prostate Specific Membrane Antigen

Figure 2

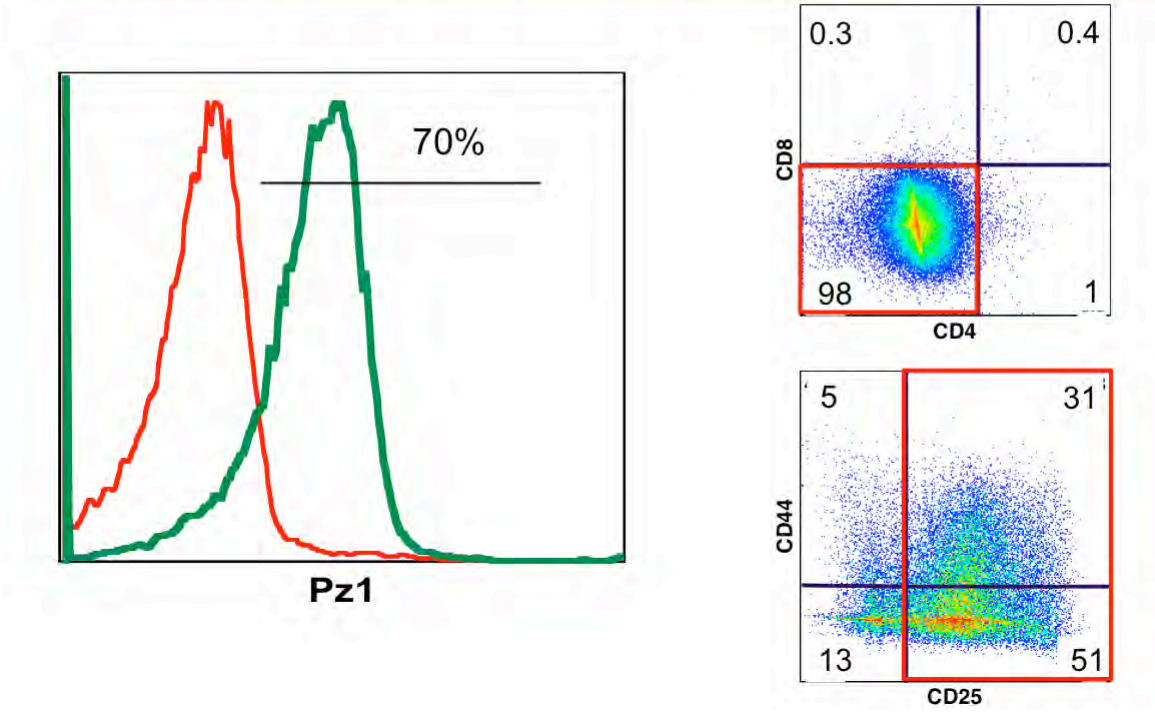

Figure 2: PreT cells primarily at the CD4-CD8-(DN) stage, expressing Pz1 gene, can be generated ex vivo. (Left panel) The co-cultured preT cells were stained with idio-specific antibody directed to the CAR, analyzed 7 days post transduction. (Right panels) The phenotype of transduced and expanded preT cells analyzed by FACS at day 21 of co-culture.

Figure 3

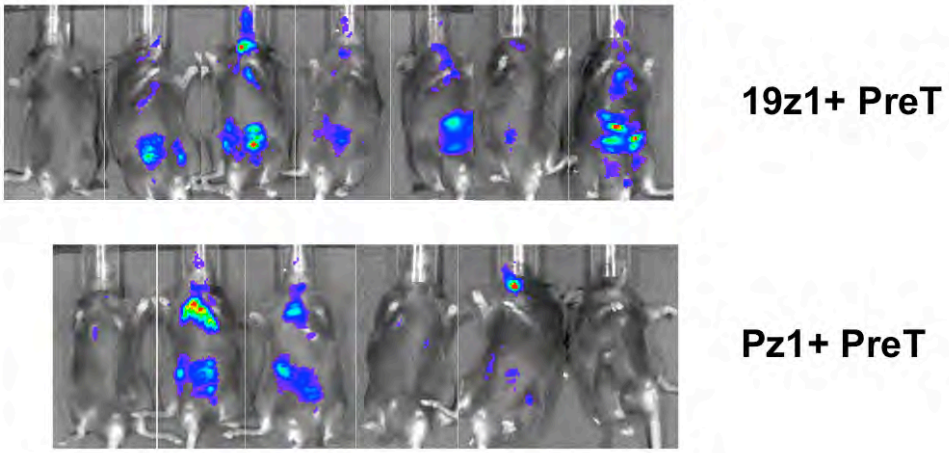

Figure 3: Pz1+ preT cells home into the thymic and extrathymic locations (A) (Pz1 or control 19z1)-luc+CAR+PreT + B6 BM → B6. In vivo imaging performed on day 14 post transplant.

Figure 4

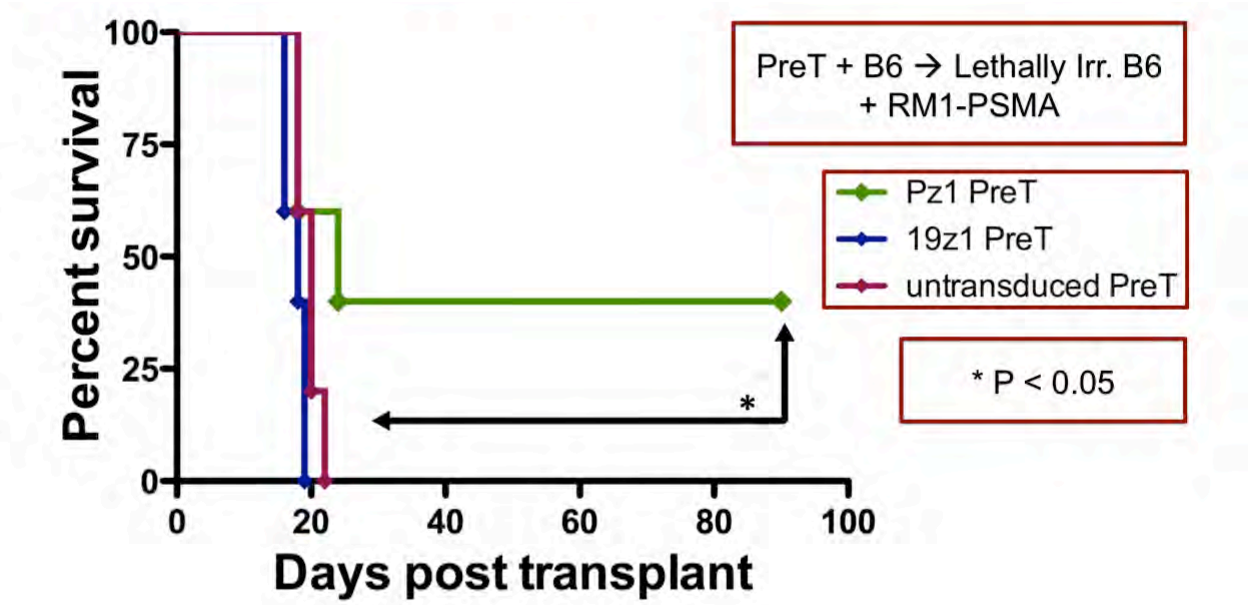

Figure 4: Pz1+ PreT cells can enhance survival of prostate cancer bearing recipients.